OMEGA bio-tek

Innovations in nucleic acid isolation

Product Manual

E-Z 96 FastFilter Plasmid Kit

D1097-00	1 x 96 preps
D1097-01	4 x 96 preps
D1097-02	20 x 96 preps

May 2013

For research use only.Not intended for diagnostic testing.

E-Z 96 FastFilter Plasmid DNA Kit

Table of Contents

Manual Revision: May 2013

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Introduction

The E-Z 96 family of products is an innovative system that radically simplifies extraction and purification of nucleic acids from a variety of sources. Key to the system is Omega Bio-tek's proprietary HiBind® matrix that avidly, but reversibly, binds DNA or RNA under optimized conditions allowing proteins and other contaminants to be removed. Nucleic acids are easily eluted with deionized water or low salt buffer.

The E-Z 96 FastFilter Plasmid DNA Kit combines the power of HiBind® technology with the time-tested consistency of alkaline-SDS lysis of bacterial cells to deliver high-quality plasmid DNA. By using the E-Z 96 DNA Plate, up to 96 samples can be simultaneously processed in less than 90 minutes. The E-Z 96 Lysate Clearance Plate obviates time-consuming centrifugation for the clearing of bacterial alkaline lysates. It also has an average DNA recovery rate 10 to 30% higher than the manual centrifugation method. Although yields vary according to plasmid copy number, *E. coli* strain, and growth conditions, a 1 mL overnight culture in LB medium typically produces 10-15 µg high copy plasmid DNA.

New In this Edition:

- HB Buffer has been replaced by HBC Buffer. Isopropanol is required and supplied by the user.
- Equilibration Buffer is no longer included with this kit. An optional Column Equilibration Protocol has been added to the protocol for your convenience.
- Equilibration Buffer is replaced with 3M NaOH provided by the user.
- 2 mL Collection Plates are now called 96-well Square-well Plates. This is a name change only, there has been ***no change*** to the plastic ware.
- 500 µL Collection Plates are now called 96-well Microplates. This is a name change only, there has been ***no change*** to the plastic ware.

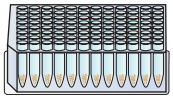
Yield and Quality of DNA

Determine the absorbance of an appropriate dilution (20- to 50-fold) of the sample at 260 nm and then at 280 nm. The DNA concentration is calculated as follows:

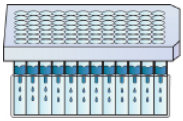
DNA concentration = Absorbance 260 × 50 × (Dilution Factor) µg/mL

A ratio greater than 1.8 indicates greater than 90% nucleic acid. Alternatively, quantity (as well as quality) sometimes can be determined best by agarose gel/ethidium bromide electrophoresis by comparison to DNA samples of known concentrations. Typically, the majority of the DNA eluted is in monomeric supercoil form, though concatemers may also be present.

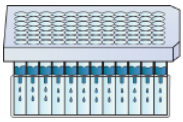
Spin Protocols



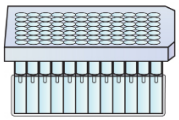
Resuspend
Lyse
Neutralize



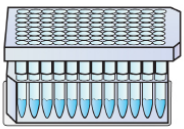
Clear lysate with
Lysate Clearance Plate



Bind and
Wash Twice

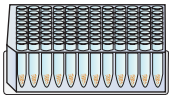


Dry Membrane

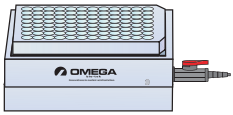


Elute

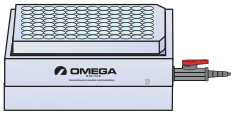
Vacuum Protocol



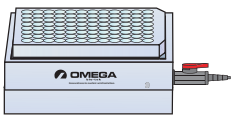
Resuspend
Lyse
Neutralize



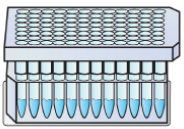
Clear lysate with
Lysate Clearance Plate



Bind and
Wash Twice



Dry Membrane



Elute

Kit Contents

E-Z 96 FastFilter Plasmid DNA Kit	D1097-00	D1097-01	D1097-02
Preps	1 x 96	4 x 96	20 x 96
E-Z 96 DNA Plate	1	4	20
96-well Square-well Plate (2.2 mL)	1	2	4
96-well Microplate (500 µL)	1	4	20
Sealing film	3	12	60
E-Z 96 Lysate Clearance Plate	1	4	20
Solution I	30 mL	110 mL	550 mL
Solution II	30 mL	110 mL	550 mL
Solution III	40 mL	150 mL	750 mL
HBC Buffer	40 mL	160 mL	3 x 270 mL
DNA Wash Buffer	30 mL	120 mL	3 x 200 mL
Elution Buffer	20 mL	80 mL	300 mL
RNase A	100 µL	400 µL	2 mL
User Manual	✓	✓	✓

Storage and Stability

All of the -Z 96 FastFilter Plasmid Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. Solution I (once RNase A is added) should be stored at 2-8˚C. All other materials should be stored at room temperature. Solution II must be tightly capped when not in use.

1. Add the vial of RNase A to the bottle of Solution I and store at 2-8˚C.

2. Dilute DNA Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D1097-00	120 mL
D1097-01	480 mL
D1097-02	800 mL per bottle

3. Dilute HBC Buffer with isopropanol as follows and store at room temperature.

Kit	Isopropanol to be Added
D1097-00	16 mL
D1097-01	63 mL
D1097-02	109 mL per bottle

4. Check Solution II and Solution III for precipitation before use. Redissolve any precipitation by warming to 37˚C.

Cleaning of 96-well Square-well Plates

The 96-well Square-well Plates supplied with this kit are reusable. To avoid cross-contamination, rinse the plates thoroughly with tap water after each use. Soak the plates in 0.5M HCl for 5 minutes then wash thoroughly with distilled water. The 96-well Square-well Plates also can be autoclaved following washing.

Guideline for Vacuum Manifold

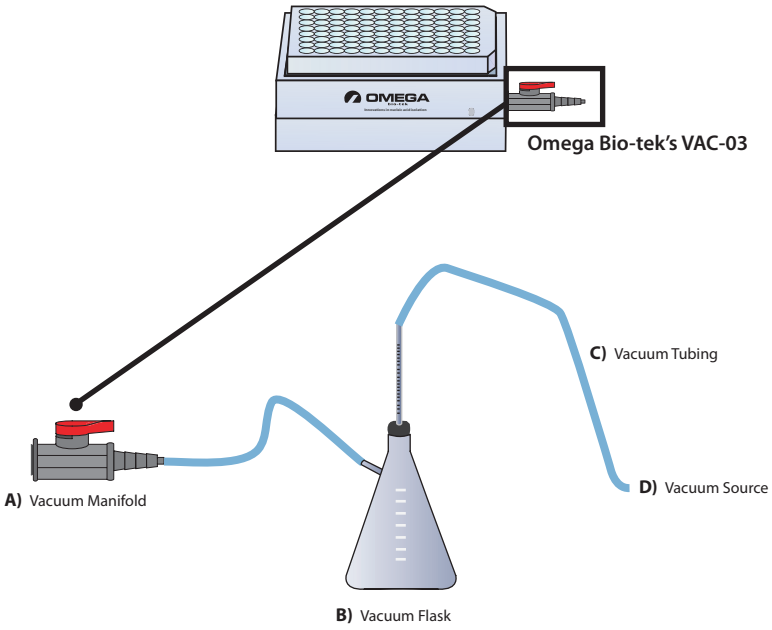
The following is required for use with the Vacuum Protocol:

A) Vacuum Manifold (We recommend Omega Bio-tek's VAC-03)
Other Compatible Vacuum Manifolds: Qiagen QIAvac24, Sigma AldrichVM20, Promega Vacman®, or manifold with standard Luer connector
B) Vacuum Flask
C) Vacuum Tubing
D) Vacuum Source (review tables below for pressure settings)

Manifold	Recommended Pressure (mbar)
VAC-03	-200 to -400

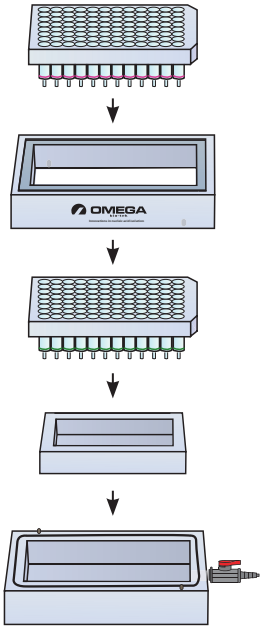
Conversion from millibars:	Multiply by:
Millimeters of Mercury (mmHg)	0.75
Kilopascals (kPa)	0.1
Inches of Mercury (inchHg)	0.0295
Torrs (Torr)	0.75
Atmospheres (atmos)	0.000987
Pounds per Square Inch (psi)	0.0145

Illustrated Vacuum Setup

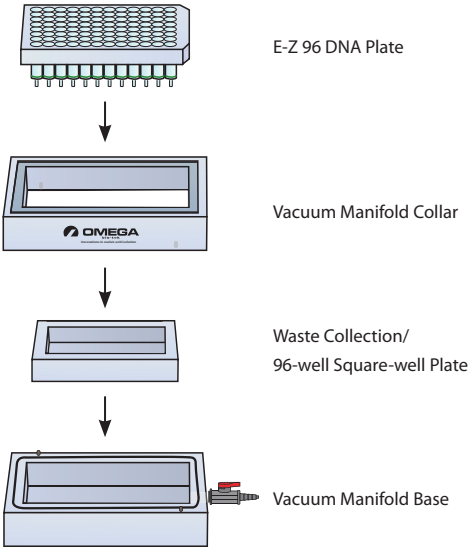


Guideline for Vacuum Manifold

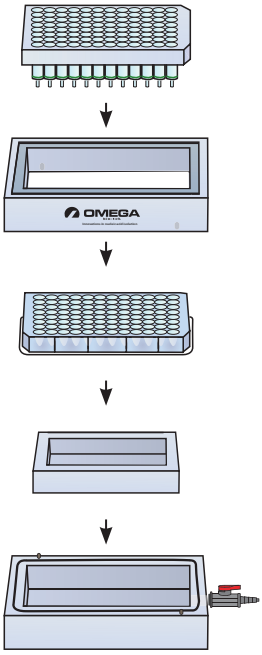
Lysate Clearance Setup



DNA Bind & Wash Setup



Standard Elution Setup



E-Z 96 FastFilter Plasmid DNA Kit - Vacuum Protocol

All centrifugation steps used are performed at room temperature.

Materials and Equipment to be Supplied by User:

- Centrifuge with swing bucket rotor capable of 4,000 x *g*
- Rotor adapter for 96-well Square-well Plates
- Standard vacuum manifold (Omega Bio-tek Cat# VAC-03 recommended)
- Optional: Vacuum oven or incubator capable of 70°C
- Optional: Sterile deionized water

Before Starting:

- Prepare Solution I, DNA Wash Buffer, and HBC Buffer according to "Preparing Reagents" section on Page 5
- Optional: Set the oven or incubator to 70°C

1. Grow 1.0-1.5 mL *E. coli* LB cultures in a 96-well Square-well Plate (2.2 mL) (provided) at 37°C with agitation (180-300 rpm) for 20-24 hours.

   Note: It is strongly recommended that an endA negative strain of *E. coli* be used for routine plasmid isolation. Examples of such strains include DH5α® and JM109®.

2. Seal the plate with sealing film.

3. Centrifuge at 1,500-2,000 x *g* for 5 minutes at room temperature.

4. Remove the sealing film and discard the supernatant.

5. Dry the plate by placing upside down on a paper towel to remove excess media.

6. Add 250 µL Solution I/RNase A. Pipet up and down to completely resuspend the cell pellet.

   Note: RNase A must be added to Solution I prior to use. Please see Page 5 for instructions.

7. Add 250 µL Solution II. Mix by gently shaking and rotating the plate for 1 minute to obtain a cleared lysate. A 2-3 minute incubation at room temperature may be necessary.

   Note: Avoid vigorous mixing as doing so will shear chromosomal DNA and lower plasmid purity. Store Solution II tightly capped when not in use.

8. Add 350 µL Solution III. Dry the top of the plate with a paper tower. Seal the plate with sealing film. Invert the plate gently 5-6 times until a flocculent white precipitate forms.

Optional Protocol for Plate Equilibration:

1. Prepare the vacuum manifold according to manufacturer's instructions. For Omega's VAC-03 manifold, set up the manifold as follows:
   a. Place the Waste Collection container inside the Vacuum Manifold Base.
   b. Place the Vacuum Manifold Collar squarely over the base.
   c. Place the E-Z 96 DNA Plate over the Vacuum Manifold Collar.
   d. Seal the unused wells with sealing film.
2. Add 100 µL 3M NaOH to each well.
3. Turn on the vacuum source to draw the NaOH through the plate.
4. Turn off the vacuum.
5. Remove the sealing film.
6. Set the E-Z 96 DNA Plate aside and disassemble the manifold.

9. Set up the manifold for Lysate Clearance as follows (refer to Page 7 for illustrations):

   a. Place the Waste Collection container into the Vacuum Manifold Base.
   b. Place the E-Z 96 DNA Plate on top of the Waste Collection container.
   c. Place the Vacuum Manifold Collar squarely over the base.
   d. Place the E-Z 96 Lysate Clearance Plate over the Vacuum Manifold Collar.
   e. Seal the unused wells with sealing film.

10. Immediately transfer the lysate from Step 9 to the E-Z 96 Lysate Clearance Plate.

11. Let sit for 5 minutes. The white precipitate should float to the top.

12. Turn on the vacuum until all the lysate passes through the E-Z 96 Lysate Clearance Plate.

13. Turn off the vacuum.

14. Discard the E-Z 96 Lysate Clearance Plate.

15. Carefully transfer the E-Z 96 DNA Plate containing the cleared lysate onto the vacuum manifold collar (refer to Page 7 for illustrations).

16. Seal the unused wells of E-Z 96 DNA Plate with sealing film.

17. Turn on the vacuum until all the lysate passes through the E-Z 96 DNA Plate.

18. Turn off the vacuum.

19. Add 500 µL HBC Buffer to each well.

    Note: HBC Buffer must be diluted with isopropanol before use. Please see Page 5 for instructions.

20. Turn on the vacuum until all the HBC Buffer passes through the E-Z 96 DNA Plate.

21. Turn off the vacuum.

22. Add 750 µL DNA Wash Buffer to each well.

    Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 5 for instructions.

23. Turn on the vacuum until all the DNA Wash Buffer passes through the E-Z 96 DNA Plate.

24. Turn off the vacuum.

25. Repeat Steps 22-24 for a second DNA Wash Buffer wash step.

26. Centrifuge at 3,000-5,000 x *g* for 15 minutes to dry the plate.

    Note: It is important to dry the plate membrane before elution. Residual ethanol may interfere with downstream applications.

27. Remove the E-Z 96 DNA Plate from the vacuum manifold. Vigorously tap the plate on a stack of paper towels until no liquid comes out. Remove any residual moisture from the tips of the E-Z 96 DNA Plate with a clean paper towel.

Optional: Place the E-Z 96 DNA Plate into a vacuum oven or incubator set at 70°C for 10 minutes to further dry the plate.

28. Return the E-Z 96 DNA Plate back onto the vacuum manifold collar.

29. Turn on the vacuum for an additional 5 minutes. This step will remove any remaining ethanol from the membrane.

30. Turn off the vacuum.

31. Remove the E-Z 96 DNA Plate and the vacuum manifold collar.

32. Set up the manifold for Elution as follows (refer to Page 7 for illustrations):

    a. Place the Waste Collection container into the Vacuum Manifold Base.
    b. Place a 96-well Microplate (500 µL) (provided) on top of the Waste Collection container.
    c. Place the Vacuum Manifold Collar squarely over the base.
    d. Place the E-Z 96 DNA Plate over the Vacuum Manifold Collar.
    e. Seal the unused wells with sealing film.

33. Add 100-150 µL Elution Buffer (10 mM Tris-HCl, pH 8.5) or sterile deionized water to each well.

34. Let sit for 2 minutes at room temperature.

35. Turn on the vacuum for 5-10 minutes to elute the DNA from the plate.

36. Turn off the vacuum.

37. Disassemble the vacuum manifold, remove the 96-well Microplate containing the eluted DNA, and seal with caps or sealing film (not provided).

38. Store at -20°C.

E-Z 96 FastFilter Plasmid DNA Kit - Centrifugation Protocol

All centrifugation steps used are performed at room temperature.

Materials and Equipment to be Supplied by User:

- Centrifuge with swing bucket rotor capable of 4,000 x *g*
- Rotor adapter for 96-well Square-well Plates
- 96-well deep-well plates
- Optional: Vacuum oven or incubator capable of 70°C
- Optional: Sterile deionized water

Before Starting:

- Prepare Solution I, DNA Wash Buffer, and HBC Buffer according to "Preparing Reagents" section on Page 5
- Optional: Set the oven or incubator to 70°C

1. Grow 1.0-1.5 mL *E. coli* LB cultures in a 96-well Square-well Plate (2.2 mL) (provided) at 37°C with agitation (180-300 rpm) for 20-24 hours.

   Note: It is strongly recommended that an endA negative strain of *E. coli* be used for routine plasmid isolation. Examples of such strains include DH5α® and JM109®.

2. Seal the plate with sealing film.

3. Centrifuge at 1,500-2,000 x *g* for 5 minutes at room temperature.

4. Remove the sealing film and discard the supernatant.

5. Dry the plate by placing upside down on a paper towel to remove excess media.

6. Add 250 µL Solution I/RNase A. Pipet up and down to completely resuspend the cell pellet.

   Note: RNase A must be added to Solution I prior to use. Please see Page 5 for instructions.

7. Add 250 µL Solution II. Mix by gently shaking and rotating the plate for 1 minute to obtain a cleared lysate. A 2-3 minute incubation at room temperature may be necessary.

   Note: Avoid vigorous mixing as doing so will shear chromosomal DNA and lower plasmid purity. Store Solution II tightly capped when not in use.

8. Add 350 µL Solution III. Dry the top of the plate with a paper tower. Seal the plate with sealing film. Invert the plate gently 5-6 times until a flocculent white precipitate forms.

Optional Protocol for Plate Equilibration:

1. Place a E-Z 96 DNA Plate on to a 96-well deep-well plate (not provided).
2. Add 100 µL 3M NaOH to each well.
3. Centrifuge at 3,000-5,000 x *g* for 3 minutes.
4. Set the E-Z 96 DNA Plate aside, discard the filtrate, and reuse the 96-well deep-well plate.

9. Place a E-Z 96 Lysate Clearance Plate onto a 96-well deep-well plate (not provided).

10. Transfer all of the lysate from Step 8 to the E-Z 96 Lysate Clearance Plate.

11. Let sit for 2-3 minutes at room temperature. A white precipitate should float to the top.

12. Centrifuge at 3,000 x *g* for 5 minutes.

13. Discard the E-Z 96 Lysate Clearance Plate.

14. Place the E-Z 96 DNA Plate on top of the 96-well Square-well Plate (provided).

15. Transfer the cleared cell lysate into the E-Z 96 DNA Plate.

16. Centrifuge at 3,000 x *g* for 5 minutes.

17. Discard the filtrate and reuse the 96-well Square-well Plate.

18. Add 500 µL HBC Buffer to each well.

    Note: HBC Buffer must be diluted with isopropanol before use. Please see Page 5 for instructions.

19. Centrifuge at 3,000 x *g* for 5 minutes.

20. Discard the filtrate and reuse the 96-well Square-well Plate.

21. Add 750 µL DNA Wash Buffer to each well.

    Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 5 for instructions.

22. Centrifuge at 3,000 x *g* for 10 minutes.

23. Discard the filtrate and reuse the 96-well Square-well Plate.

24. Repeat Steps 21-23 for a second DNA Wash Buffer wash step.

25. Remove the E-Z 96 DNA Plate and vigorously tap the plate on a stack of paper towels until no liquid comes out. Remove any residual moisture from the tips of the E-Z 96 DNA Plate with a clean paper towel.

Optional: Place the E-Z 96 DNA Plate into a vacuum oven or incubator set at 70°C for 10 minutes to further dry the plate.

26. Place the E-Z 96 DNA Plate onto a 96-well Microplate (500 µL) (provided).

27. Add 100-150 µL Elution Buffer (10 mM Tris-HCl, pH 8.5) or sterile deionized water to each well.

28. Let sit for 2 minutes at room temperature.

29. Centrifuge at 3,000 x g for 5 minutes.

30. Seal the 96-well Microplate with caps or sealing film (not provided).

31. Store at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff , toll free, at **(800-832-8896).**

Possible Problems and Suggestions

Problem	Cause	Solution
Low DNA yields	Poor cell lysis	Only use LB or YT medium containing antibiotic(s). Do not use more than 2 mL culture with high copy plasmids. Cells may not be dispersed adequately prior to addition of Solution II. Mix cell suspension to completely disperse. Increase incubation time with Solution II to obtain a clear lysate. Solution II, if not tightly closed, may need to be replaced.
	Bacterial culture overgrown or not fresh	Reduce quantity of starting material.
	Low copy number plasmid used	Low copy number plasmids may yield as little as 0.1 µg DNA from a 1 mL overnight culture. Increase culture volume to 3 mL.
Problem	**Cause**	**Solution**
No DNA eluted	DNA Wash Buffer not diluted with ethanol	Prepare DNA Wash Buffer according to instructions on Page 5.
	HBC Buffer not diluted with isopropanol	Prepare HBC Buffer according to instructions on Page 5.
Problem	**Cause**	**Solution**
High molecular weight DNA contamination of product	Over mixing of cell lysate upon addition of Solution II.	Do not vortex or mix aggressively after adding Solution II. Adequate mixing is obtained by simply inverting and rotating the plate.

Problem	Cause	Solution
Optical densities do not agree with DNA yield on agarose gel	Trace contaminants eluted from column increase A_{260}	Make sure to wash plate as instructed. Alternatively, rely on agarose gel/ethidium bromide electrophoresis for quantization.
Problem	**Cause**	**Solution**
RNA visible on agarose gel	RNase A not added to Solution I	Add 1 vial of RNase to each bottle of Solution I
Problem	**Cause**	**Solution**
Plasmid DNA floats out of well while loading agarose gel	Ethanol not completely removed from column following wash steps	Follow the optional drying step to completely dry the HiBind® matrix.
Problem	**Cause**	**Solution**
Plasmid DNA will not perform in downstream application	Traces of ethanol remain on column prior to elution	The E-Z 96 DNA Plate must be washed with 100% ethanol and dried before elution. Ethanol precipitation may be required following elution.
	DNA is permanently denatured	Cell lysis process should not be over 5 minutes. It may cause DNA to permanently denatured.

Ordering Information

The following components are available for purchase separately.
(Call Toll-Free Number (1-800-832-8896)

Product	Part Number
Solution I (250 mL)	PS001
Solution II (250 mL)	PS002
Solution III (250 mL)	PS003
Elution Buffer (100 mL)	PDR048
DNA Wash Buffer (100 mL)	PS010
RNase A (400 µL)	AC117
EZ 96 DNA Plate (10)	BD96-01
Sealing Film (100/box)	AC1200-01

Notes: